Decoding the silent walk of COVID-19: Halting its spread using old bullets

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ABSTRACT

Severe acute respiratory syndrome (SARS) develops within 3–14 days when CoV2 invades epithelial, myeloid cells in the nasopharynx and pneumocytes in the respiratory tract through angiotensin converting enzyme (ACE2). Infection swiftly disseminates to gastrointestinal, cardiovascular, renal organs as well as immune system to deregulate their normal functioning through unique and distinct mechanisms. The health system and economy has been intensely thwarted by the rapid spread and exorbitant mortality caused by COVID-19 disease across the globe. The acute progression of the disease and high infection rate pose an enormous challenge for its therapeutic management and critical care. The viral structure, genome and proteome have been deciphered which yielded cues for targeting already available therapeutic entities. More than 200 compounds have been screened and till date approximately 69 therapeutic agents are undergoing clinical trials across the world. Among these, remedesivir (RMD), chloroquine (CQ), hydroxychloroquine (HCQ), noscapine (NOS) and heparin have demonstrated fairly promising results in preclinical and clinical studies. Recently, RMD has been approved by USFDA for the management of COVID 19. However, intense research is going on to screen and ace the 'magic bullets' for the management of SARS-CoV2 infection worldwide. The current review illustrates the plausible therapeutic targets in SARS-CoV2 important for inhibition of virus cycle. In addition, the role of RMD, CQ, HCQ, NOS and heparin in combating infection has been addressed. The importance of vitamin C and D supplements as adjunct therapies in the prevention of SARS-CoV2 virus infection have also been summarized.

1. Background

COVID 19 results in severe acute respiratory distress and immune system furor cumulating in multi-organ failure in almost 5–20 % cases. The disease can be grossly asymptomatic (80 % asymptomatic carriers) or demonstrates mild to severe devastating effects in affected population [1]. SARS-CoV-2 infection cases were initially traced in Wuhan city, located in Hubei province of China on December 31, 2019. Since its spread to 215 countries, more than 31 million infection cases have been recorded worldwide with 965 K deaths till date. The catastrophic spread of disease has brought extreme personal, societal, economic, medical and industrial impact. COVID19 has forced the human civilizations to think, innovate, develop and prioritize all possible strategies to combat the infection in last 10 months. Therefore, World Health Organization (WHO) in coordination with global expert network is working rigorously to analyze the available epidemiological data, clinical histories and devising treatment strategies to form guidelines for controlling the infection.

SARS-CoV-2, the etiological agent for COVID19 pandemic was initially isolated from a Chinese patient and its genome sequence was provided to researchers by January 7, 2020 [2]. It suggested that CoV2 is an enveloped positive sense RNA virus belonging to β-coronavirus subfamily. Bats have been identified as the potential primary harbourers of SARS-CoV2 along with some of the bat predators which express virus receptors on their respiratory cells and serve as intermediate/reservoir host for virus transmission. The first human transmission of novel corona virus has been indicated to occur from Pangolin or from Bats, the key reservoirs of CoV_RaTG13 [3]. Interestingly, SARS-CoV2 and CoV_RaTG13 have identical genomic regions owing to 96.2 % homology w.r.t Spike (S) protein [4–7]. Previously, bats were also identified to be

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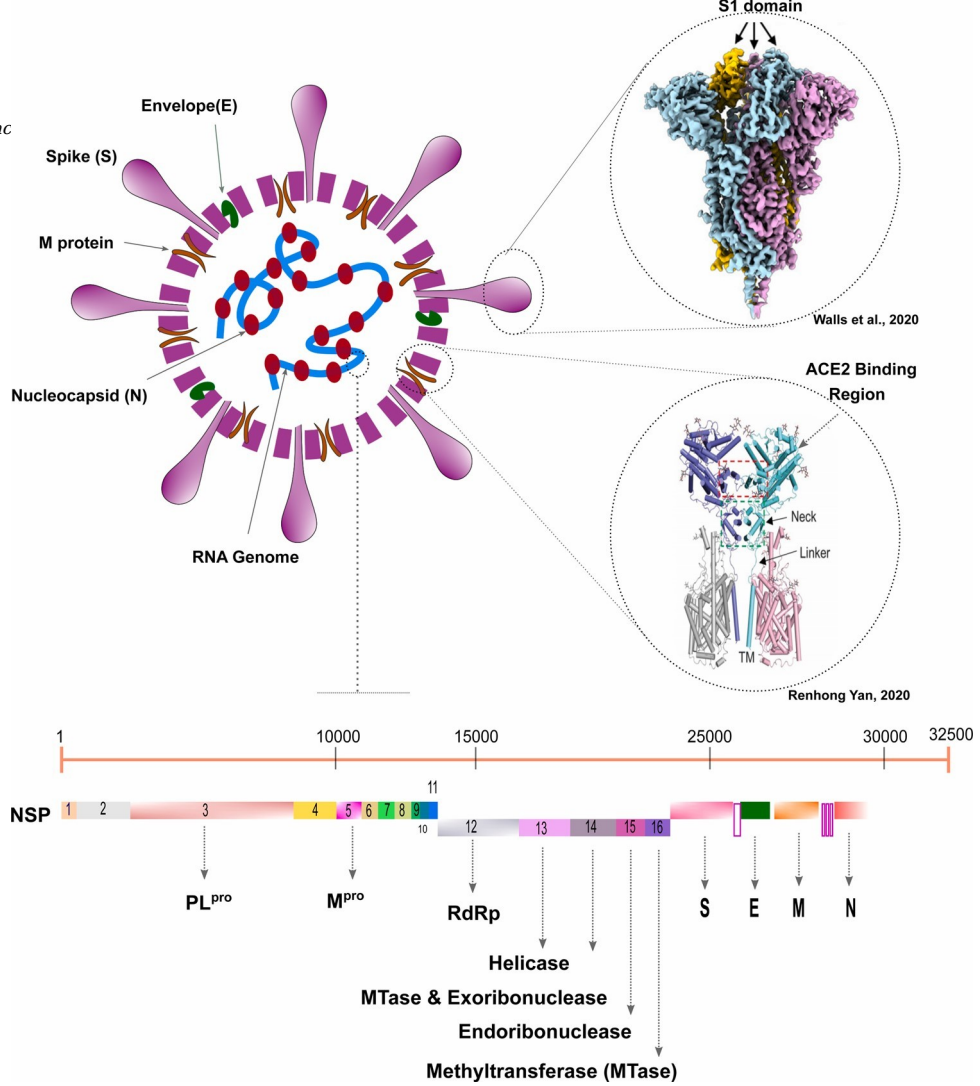

Fig. 1. SARS-CoV2 structural, functional and genomic composition, [A] Four major structural proteins S, M, E, N and RNA genome; [B] Functional elements of S protein (PDB: 6VXX) and ACE2 (PDB: 6M17) required for virus entry and [C] Composition of RNA genome encoding various structural and non-structural proteins (NSP).

natural reservoirs for a wide variety of COVs including SARS-COV and Middle East respiratory syndrome coronavirus (MERS-COV) [8–10]. The origin of ongoing COVID-19 pandemic has been traced to Chinese sea food market located in Wuhan city [11]. Owing to the high stability of SARS-CoV-2 viruses in air/fomites as well as on inanimate surfaces and human bodies, the probability of transmission is high either through inhalation, inadvertent contact with body fluids or other contaminated sources [12,13]. Respiratory air droplets and fomites are the main mode of human-to-human transmission. The virus can be present in aerosol for 3 h and infect upto 10 m in closed rooms [12].

The best clinical management strategies employed in the hospital settings across the world could not show decrement in the mortality rate. In a desperate need to manage the disease repurposing of clinically approved drugs is the sole option available with clinic's armory at present. Moreover, the tested drug combinations have demonstrated mixed non-uniform results in the affected patients. Therefore, a need to extend the repertoire of drugs or adjunct therapeutics to deal with COVID19 is warranted. In the present review, the underlying mechanisms for establishment of infection and pathogenesis of SARS-COV-2 have been discussed in elaborative manner. In addition, approaches for the development of new therapeutic modalities have been proposed. We have reviewed literature in support of already available nucleotide analogues (For instance, remedesivir, RMD), chloroquine (CQ), hydroxychloroquine (HCQ), noscapine (NOS) and heparin in context to their extended usage for combating COVID-19.

2. COVID-19/SARS-CoV-2: structure, functions and plausible drug targets

SARS-CoV2, an enveloped positive strand RNA virus (~30 kb) enters the human host through inhalation or contact [13]. Upon entry into human host, the virus primarily infects the epithelial cells of salivary gland, upper respiratory tract and then further transgresses to infect the lower respiratory tract, liver, kidney, heart etc. The entry of virus in the mammalian cells is maneuvered by a coordinated interplay between viral proteins, recipient cell membrane proteins and the cellular proteases.

Angiotension converting enzyme 2 (ACE2) present on type I and type II pneumocytes towards the apical surface acts as receptor for attachment of SARS-CoV2 virus [14,15]. The viral spike protein (S protein) performs the pivotal role of binding to the extracellular domain of ACE2 [16]. The peptidase domain in ACE2 and S1 domain in S protein are essential for mediating direct interaction (Fig. 1). S2

domain adjoining S1 in the native S protein masks ACE2 binding region of S1.

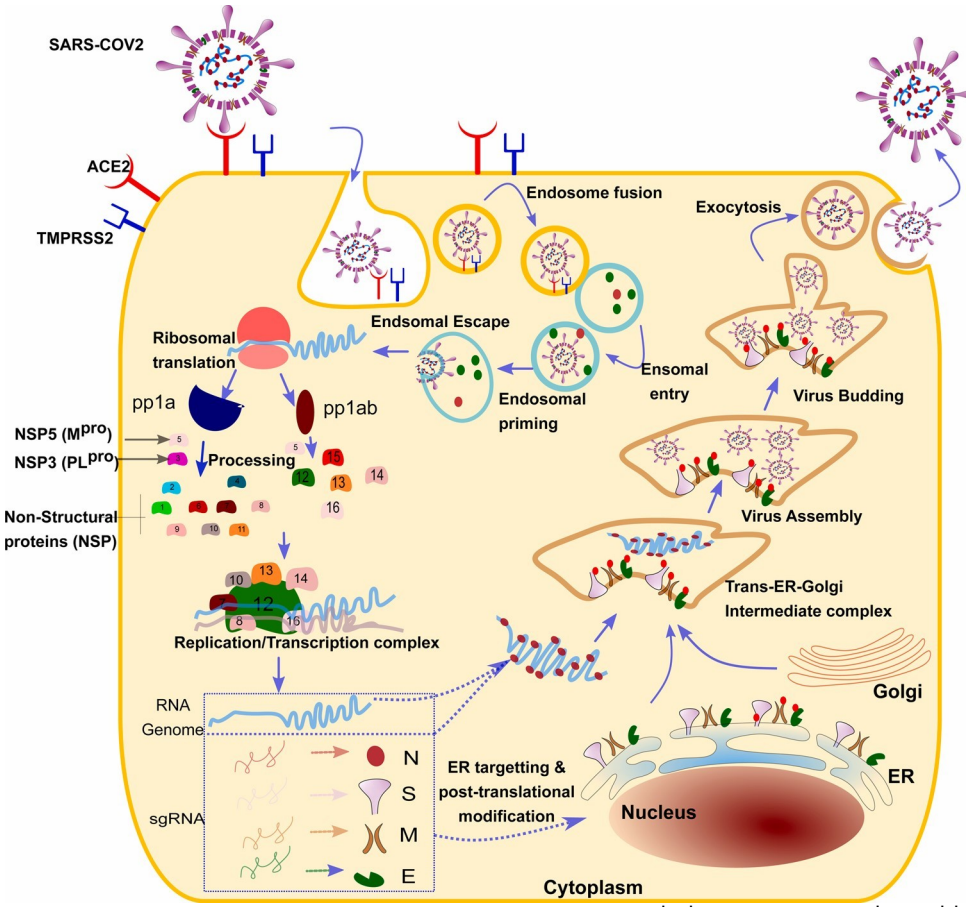

Fig. 2. Mechanistic overview of virus entry, assembly and its exit in mammalian cell. SARS-CoV2 enters the cell using its glycosylated S protein and host ACE2 interaction, its voyage in endocytic vesicle, endocytic priming and the endocytic escape occurs through a well orchestrated maneuvering of host enzymes followed by smart use of host transcriptional machinery and post translational mechanisms for a complete virus assembly. Finally the externalization of viral particles to enter the cells in the vicinity is shown as graphics.

Consequently, proteolytic cleavage between S1 and S2 is prerequisite for liberating S1 domain to facilitate its interaction with the peptidase domain of ACE2, while S2 domain still remains attached to the complex in a pre-fusion state. Interestingly, SARS-CoV2 has been found to acquire a unique cleavage site between S1 and S2 which is recognized by the host furin protease [17]. Widespread expression of furin protease in various tissues [18,19] is proposed to be a key factor in conferring tissue specific viral tropism. The interference in the viral attachment machinery through development/identification of lead molecules which prominently target the S1 domain and ACE2 peptidase domain or their interaction, can be a wise pharmaceutical approach to prevent SARS-CoV2 infection. SARS-CoV2 interaction with ACE2 results in internalization of the viral-ACE2 complex through receptor mediated endocytosis. The internalization of the virus can also take place alternatively/additionally through yet to be deciphered process, independent of clathrin and caveolin (Fig. 2) [20]. Whether such process is active in SARS-CoV2 cellular entry is still a matter of debate. Prior to fusion of virus laden vesicle to the endosome, virus primes itself andacquires the functionality to exit endosome. In the process, TMPRSS2, a trans-membrane host serine-protease trims S2 domain of spike protein [21]. An additional cleavage in S2 domain is facilitated by Cathepsin-L in the endosomal compartment which finally unmasks the endosomal membrane fusion domain located in S2 to mediate its endosomal escape [22]. Although no study has been conducted so far for SARS-CoV2 entry through this process, but evolutionary conservation with SARS-CoV signifies the existence of similar mechanism. Therefore, inhibition of TMPRSS2 and Cathepsin-L may also be vital therapeutic targets to halt endosomal escape of SARS-CoV2.

Endosomal escape results in release of viral RNA in the cytoplasm, where N protein can also be a key mediator in preventing the activation of antiviral response of host cells. This feature is embodied in the RNA binding properties of N protein. 200–400 copies of N protein are found to adorn the complete RNA genome of the virus. It serves as primary barrier in preventing interaction of the ‘exogenous RNA’ sensing mechanism of the host cells. One such mechanism is Dicer-RISC mediated anti-viral defense. In eukaryotic cells, under normal healthy conditions, the entry of the foreign double stranded RNA (dsRNA) triggers its recognition by Dicer which processes it to 20–25 bp dsRNAs by cleavage phenomenon [23]. The thermodynamically unstable strand of this 20–25bp dsRNA, then gets incorporated in RISC complex [24]. Using this strand, RISC complex recognizes the invading RNA by complementary base pairing and performs a cleavage reaction in the foreign RNA to render it non-functional. In case of SARS-CoV 2, N protein has unique functionality of preventing action of DICER-RISC machinery through direct binding to their substrate at the stage of pre-DICER or post DICER processing stage [25]. In light of the established facts, pharmacological interventions interfering with N protein-RNA genome interaction may in turn

increase the susceptibility of RNA genome for recognition by RNA sensing machinery to mediate its clearance.

The downstream viral replication process is tightly regulated by complex interplay between viral machinery and the host cell proteome. The process is mediated through formation of a transcription complex constituted mainly by NSP1-16 associated with proteolytic digests of polyprotein complexes (pp1a, pp1ab). The proteolytic digestion of these polyprotein complexes takes place through two proteases viz. main protease (M^{pro}, also called as $3CL^{pro}$) and papain like protease (PP^{pro}) [26,27]. Unique mutations gained by SARS-CoV2 have been found to enhance the catalytic efficiency of M^{pro} [28]. This can also be one of the contributing factors in accentuating the viral pathogenicity. Further to initiate negative RNA strand synthesis, NSP12 (RNA dependent RNA polymerase, RdRP) utilizes its intrinsic polymerase activity and recruits various regulatory factors for this process. RdRP interaction with NSP7 and NSP8 enhance its polymerase activity [29]. The helicase activity of NSP13 required for RNA synthesis also gets pronounced upon association with RdRP [30]. Moreover, NSP10 interaction with NSP14 and NSP16 has been found to be an essential event for activating NSP14 exoribonuclease activity and NSP-16 Mtase (methyltransferase) activity which are important determinants of viral RNA synthesis [31]. Other accessory mediators such as NSP1, NSP2, NSP4, NSP6, NSP9, and NSP11 are generally not essential for virus viability but aid in the viral replication/transcription process [32,33]. Therefore, targeting the essential machinery of viral replication is an interesting avenue to control SARS-CoV2 infection load.

The cumulative response of the replication/transcription complex results in production of a large RNA genome (~30 kb) as well as synthesis of sub-genomic RNA (sgRNA). sgRNA then gets translated to yield various structural and non-structural proteins for virion packaging. Microtubule based transport system can play a key role in facilitating the directional trafficking of newly synthesized viral proteins; hence any direct intervention in such transportation may compromise the virus assembly processes [34]. A similar observation has been noticed in case of porcine transmissible gastroenteritis virus (TGEV). It is reported that upon treatment with a β-tubulin depolymerising agent (Nocadazole), the S protein got dispersed throughout the cytosol. The scattered distribution of S protein was further correlated with decreased incorporation of S protein in virion and subsequently a low viral titer in nocadazole treated cells [35]. If S protein of SARS-CoV2 follows identical pattern, the pharmaceutical interventions targeting tubulin polymerization may interfere with viral assembly thereby decreasing the viral load. Curtailing S-protein incorporation in virion can also decrease its binding probability on host cells and thus can help in decreasing its pathogenicity.

The synthesized protein further undergoes processing in endoplasmic reticulum and golgi compartments. S-glycosylation of the protein is one such modification that can regulate protease accessibility as well as modify their functions. Seven glycosylation sites have been found to be located in ACE2 extracellular domain [16]. Although, inhibition of glycosylation machinery did not facilitate enhanced viral clearance but it could decrease the viral load [36]. Thus targeting glycosylation machinery can also be another avenue to exploit for increasing the efficacy of the pharmaceuticals. Furthermore, the unique properties of individual viral protein/s or their subdomains may play an essential function in posttranslational processing in addition to their classical role in viral assembly. In case of SARS-CoV, the carboxy terminal domain of N protein (Residues 366–422) mediates interaction with TRIM25 and thus prevents its further interaction with RIG-1. This leads to inhibition of TRIM25 mediated ubiquitination of RIG-1, which prevents downstream activation of RIG-1, associated type-1 interferon response [37,38]. SARS-CoV2 has 78.33 % homology in this interacting domain and may present INF-1 response in a similar fashion. The effectiveness of the IFN based therapies in combating the COVID-19 disease further substantiate the possible involvement of SARS-CoV2 N protein in down-regulating type I IFN response in the affected patients [39]. Therefore, pharmaceutical strategies that can induce type 1 IFN response can be helpful in management of COVID19. The viral proteins become ready to be packaged in a complete virion following completion of
post-translational processing. The trans-golgi intermediate compartment (ERGIC) was found to be an imperative site for the virus assembly and budding [40,41]. At this site RNA genome complexation occurs with N protein which further interacts with other structural proteins of SARS-CoV2. Among them, the interaction of cytoplamic domain of M protein with S protein is quite important for RNA genome encapsulation in the virion. Further interaction of M protein with N protein through carboxy terminal domain also aids in its assembly into virion structure [42,43].

Another structural protein that plays an essential function is envelope protein (E). E protein forms homoligomeric structures, in which homopentameric structure seems to have the property to behave as mild cation (Ca^{2+}, K^{+}, Na^{+}) selective ion channel [44–46]. The formation of such cation selective ion channel in the host plasma membrane, Trans ER-Golgi complex (ERGIC) can induce flux of ions across the membranes and thus can alter the ionic balance of the cellular and sub-cellular compartments. The Ca^{2+} ion imbalance across the ERGIC due to ion channel activity of E protein results in activation of NLRP3 inflammasome mediated IL-1β production [46]. Furthermore, E protein expression has been found to be associated with increase in NF-κβ stimulatory pathway [47]. Therefore, E protein seems to act as a mediator for exacerbated inflammatory responses through NF-κβ and IL-1β thereby increasing pathogenesis. The virus lacking E protein has been found to be severely compromised in replication. Therefore, the association of E protein expression not only relates to viral pathogenesis but also to its unique function to alter the ionic composition of sub-cellular compartments of lysosome and endoplamic reticulum which in turn can correlate well with the change in the cellular pH. Therefore, it is likely that E protein also alters the pH of cellular compartment to a level that is conducive

for viral assembly. Additionally, a low pH of the endosomal compartments is required for resident protease functionality and dissociation of virus-receptor complexes [48]. Therefore, pharmacological strategies modulating the pH of these sub-cellular compartments may provide dual advantages for prevention of viral endosomal escape as well as altering of viral packaging in ERGIC.

The assembled virus particles exit from the primary infected cells through exocytosis to enter the interstitial fluid where they can infect cells in the vicinity and then transiently enter the systemic circulation. The virus may undergo further maturation in tissue fluid or blood through a novel mechanism that may increase its infectivity. One such role is thought to be played by the blood kallikerin system. Kallikerin 13 (KL13), the component of kallikerin system is found to play an essential role in coronovirus (HCoV-HKU1) priming by mediating cleavage at S1/ S2 boundary [49]. Similar involvement of host KL13 in SARS-CoV2 priming is a matter of investigation. Normally, kallikerin acts on kininogens (High molecular weight kininogens, HMWK and Low molecular weight kininogens, LMWK) directing the synthesis of bradykinin which in turn exerts its vasodilatory response through bradykinin receptor 2 (B2). Excess of the bradykinin is associated with blood vessels leakage, tissue swelling and inflammation. In case of COVID-19, hypofunction of ACE2 dependent negative regulatory loop may further cause stimulation of bradykinin dependent downstream signalling [50,51]. A significant homology of bradykinin motif (GFSPFRSSRIG) has been found in SARS-COV S protein whose role in the viral pathogenesis is still undefined. A probable role of bradykinin in aiding the viral spread can be assumed since the antibody response to SARS-COV S protein is found to cross-react with bradykinin protein [52]. Therefore, it is probable that antibody response generated against S protein motif during SARS-CoV2 infection might be neutralized by bradykinin in the cellular system. This may aid in viral evasion manoeuvring during cellular spread. Thus, any alteration in kallikerin system functionality can influence the SARS-CoV2 propagation and can amplify the inflammatory responses. Consequently, pharmacological interventions targeting KL13 or its associated molecular signalling can be effective in dealing the current COVID-19 crisis.

Further propagation of the viral spread is associated with hypercoagulative response in severe cases of COVID-19 as indicated by an increased level of D-dimer (>1 μg/ml) [53]. The increase in D-dimer level does not correlate with thrombolysis observed in COVID patients at late stages therefore; mechanism that might be responsible for activation of this coagulative response needs to be explored further. Interestingly, dipyridamole, a FDA approved anti-coagulant was effective in the functional recovery of few severe COVID-19 cases. Other than anti-coagulation properties, its proposed interaction with M^{pro} protease of SARS-CoV2 may also be responsible for curative effects observed in affected patients [54]. Moreover, heparin was also found to possess affinity for S1 domain of S protein [55], its use can not only interfere with the viral spread but also prevent the hypercoagulation response. Thus, any intervention having the ability to restore the homeostasis of the coagulative machinery may act as a supportive therapy for COVID patients.

Table 1
Relative proportion of clinical trials conducted for different categories of established drugs in order to determine their safety and efficacy in COVID-19 patients.

Category	Subcategory	% Clinical trials
Antimalarial drugs	Chloroquine diphosphate	30
	Hydroxychloroquine	7
Antiviral drugs	Ritonavir+Lopinavir	8
	Interferon (Recombinant)	4
	Favipiravir	4
	Baloxavir	2
	Darunavir	2
	Ganovo	2
	ASC09/Ritonavir	1
	Arbidol hydrochloride	1
	Carrimycin	1
	Triazavirin	1
Corticosteroid	Methylprednisolone	4
Stem cells	Mesenchymal stem cells	9
	Mesenchymal stem cells (Umbelical cord)	1
	Umbilical cord blood plasma	1
	Blood mononuclear cells (Umbelical cord)	1
	Blood-derived stem cells	2
	Mesenchymal stem cells and NK cells	1
	Mesenchymal stem cells and NK cells	1
Immunomodulators	Recuperative plasma	11
	CMAB806 (IL-6)	1
	NLRP Inflammasome inhibitor (Tranilast)	1
Monoclonal antibodies	Tocilizumab	2
	Adalimumab	1
	Ixekizumab	1
	Camrelizumab	1
	Anti-PD-1 (Programmed cell death) therapy	1
Vaccine	Adenoviral vector vaccine	2
	Adenoviral vector vaccine	1
	Dendritic Cells vaccine	1

3. Old bullets for new target: Drugs for mitigating the symptoms of coronavirus infection

The desperate need of therapeutic entities for COVID19 has drawn the attention of scientists and clinicians world over. Since vaccine development for SARS-CoV2 is still in pipeline, the repurposing of drugs already in the armor is the best plausible approach. A plethora of anti- parasitic/anti-viral drugs, monoclonal antibodies, vaccines, stem cell therapies were tried in last nine months as part of well-designed 265 clinical trials out of which 115 trials documented direct anti- SARS- CoV2 effect depicted by reducing the viral loads or indirectly helped in management of COVID19. A summary of

percentage of clinical trials conducted on different categories of established drugs to determine the safety and efficacy in COVID-19 patients is presented in Table 1.

3.1. Nucleotide analogues

A number of antiviral nucleotide analogues such as favipir (FAV), ribavirin and remdesivir (RMD) have gained importance for treatment of SARS-CoV2 and undergoing clinical trials. FAV (originally designed for swine flu) has RNA-dependent RNA polymerase activity (RdRp) against nCoV2, has been recommended strictly for emergency use in china as it has teratogenic potential [56].

Similarly, ribavirin is being tried in combination with other antiviral agents [57]. RMD an investigational nucleotide analog prodrug, originally developed by Gilead against Ebola virus, effectively inhibits viral RNA-dependent RNA polymerase (RdRp) activity (Fig. 3). There was no consensus data for safety, adverse events and its effect on viral loads or dose-dosage regimens. Its Phase I and II studies have been completed recently [58,59]. Two phase 1 studies conducted so far evaluated the safety and pharmacokinetics (PK) of single escalating and multiple intravenous (IV) doses of RMD (solution or lyophilized formulation) in healthy subjects. Lyophilized formulation was evaluated in clinical trials due to its advantage of storage stability in resource-limited settings. All adverse events following drug administration were Grade 1 or 2 in severity. Overall, RMD exhibited a linear profile following single-dose intravenous administration (over 2 h) of its solution formulation across the dose range of 3–225 mg. Both lyophilized and solution formulations provided comparable pharmacokinetic profiles. High intracellular concentrations of the active triphosphate (approximately 220 to 370- folds higher than the *in vitro* EC50 against SARS-CoV-2 clinical isolate) were achieved following infusion of 75 mg or 150 mg lyophilized formulation over 30 min or 2 h. Following administration of multiple-doses of RMD (150 mg once daily for 7 or 14 days), the drug exhibited a pharmacokinetic profile similar to single-dose administration. Its metabolite GS-441524 accumulated approximately 1.9-fold after daily dosing. Overall, RMD exhibited favourable safety and pharmacokinetic profiles that supported once-daily dosing [60].

RMD was first used for the treatment of COVID19 patient in United States in January 2020. Thereafter, Gilead in collaboration with Chinese partners (since February 2020) conducted phase 3 randomized, double- blinded, placebo-controlled, multicenter trials to determine the safety and efficacy of RMD in hospitalized patients with severe COVID-19 [58]. The treatment was designed as 200 mg of RMD on day 1 followed by 100 mg/day for either 5 or 10 days in two separate trials (NCT04257656 &NCT04252664). A double-blind, randomized, placebo-controlled trial of intravenous RMD administered to hospitalized adult COVID-19 patients with the evidence of lower respiratory tract involvement was performed. Patients were randomly assigned to receive either RMD (200 mg loading dose on day 1, followed by 100 mg daily for up to 9 additional days) or placebo for up to 10 days. RMD was superior to placebo in shortening the time to recovery in this group of COVID-19 patients with comparatively reduced infection load in lower respiratory tract [60].

The clinical trial indicated 62 % recovery of the patients treated early as compared to 49 % of the patients who were treated late during the infection course. Gilead is scaling up production of RMD but its wide usage to treat COVID19 patients in future will depend on its safe use in co-morbid patients, adverse effects and the treatment regimens. Although RMD showed promising results against SARS-CoV2 with acceptable side-effects, scientific fraternity is still waiting for the results of clinical trial (NCT04252664). *3.2. Chloroquine and hydroxychloroquine*

Chloroquine, CQ and hydroxychloroquine, HCQ (Fig. 3) have been used extensively in the treatment of malaria for more than 70 years. CQ/ HCQ reduces the infection load by blocking the entry of viruses into mammalian cell systems [61]. Recently, it has been described to be effective in reducing the viral loads in COVID-19 patients. The virus enters the intracellular *milieu* owing to receptor mediated endocytosis. Once in the endosome, the viral particles experience a drop in pH which in turn facilitates release of viral genome into the cytoplasm. However, treatment of infected cells with CQ raises the endosomal pH which prevents viral release and blocks viral propagation [62,63]. The replication cycle/s of a number of viruses are known to be inhibited by CQ [64,65]. SARS-CoV-1 reproduction cycle was found to be inhibited following CQ treatment *in vitro* [66,67]. On similar lines, HCQ (Fig. 3) was tested in COVID-19 patients at the dose-dosage regimen of 600 mg per day for 10 days and subsequently viral loads in nasopharyngeal wash were computed on daily basis. In addition, a group of patients were co-administered azithromycin in dose-dosage regimen in order to prevent bacterial super-infection. On day 6th, RT-PCR analysis confirmed that 70 % of treated patients no longer harboured virus in comparison to 12.5 % of controls. Therefore, combination of HCQ with azithromycin was found to be more effective in reducing viral load than HCQ alone [68]. It has been found that the primary action of HCQ or CQ is because of their ability to increase the pH of the vesicles from pH 4–6. In its basic form, HCQ can readily pass through the plasma membrane and get accumulated in the endocytic vesicles. Due to low pH of the vesicles, it creates suitable environment for protonation of the basic form. In its protonated form (B^+H^+), HCQ becomes polar and hence its ability to cross the plasma membrane decreases. Owing to this event, HCQ concentration in the vesicle continuously accumulates until a steady state concentration is achieved accompanied by rise in pH of the vesicles by 1–2 pH units. Under such modified conditions, the functionality of the vesicles' acid proteases gets altered which may lead to a variety of consequences. Therefore, the efficacy of HCQ/CQ in treatment the COVID-19 cases can be mechanistically related to rise in vesicular pH.

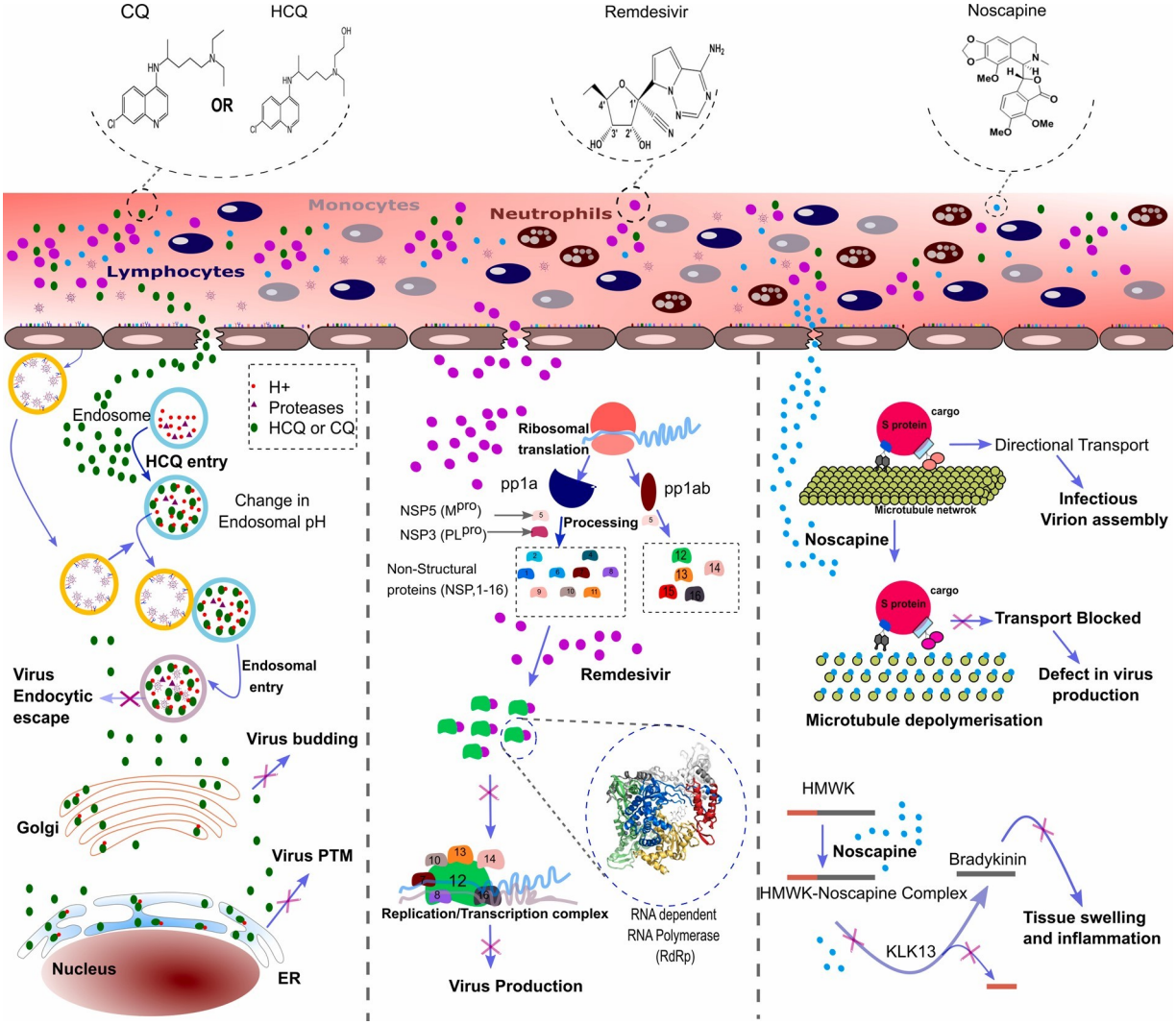

Fig. 3. Old bullets for new coronavirus: Chloroquine (CQ) or Hydroxychloroquine (HCQ), Remdesivir (RMD) and Noscapine (NOS). A compendium of mechanism of action of RMD, CQ/HCQ and NOS depicts interventions at different steps in the viral life cycle or manipulate different aspects of host physio-mechanisms with a perspective of decrease in viral load alone or associated with "correction" of virus associated host pathophysiology.

Support for this can be derived from the essential role played by the TMPRSS2 and Cathepsin-L in the escape of the SARS-COV from the endosome into the cytoplasm [16]. SARS-CoV2 also requires TMPRSS2 for its propagation, hence similar mechanism might be utilised for its endosomal escape. Therefore, HCQ mediated alteration in endosome pH might be the key interfering factor for SARS-CoV2 endosome escape pathways which needs further elucidation. A recent study has identified the endoplasmic reticulum and Golgi apparatus as site for the glycosylation of SARS-CoV2 S-protein [68]. The glycosylation event is thought to be essential for virus attachment to ACE2 and its priming in the endosome for the membrane fusion required for its cytoplasmic entry. Inhibition of the ACE2 glycosylation by CQ can successfully decrease the SARS CoV2 viral load similar to an earlier observation in case of SARS CoV infection [66]. CQ treatment may modulate ACE2 and S protein interaction that ultimately interferes with virus internalization and virus spread. Efficacy of the HCQ in treating COVID patients at the secondary stage of infection involving brain manifestations is still questionable. Since, HCQ can cross the blood brain barrier, therefore, its usage can also be extended to the patient's diagnose with the neurotransmission of the SARS-CoV2. The classical role of CQ and HCQ in inhibiting the reproduction cycle of the virus to its wide usage in Systemic lupus erythematosus (SLE) and other autoimmune diseases suggests a multipronged effect of CQ/HCQ on COVID-19 patients [69]. CQ can also inhibit complement system which has not been taken into consideration in case of COVID-19 infections. A previous study has also indicated its effect on complement pathway inhibition [70]. Therefore CQ/HCQ might act as double edged sword by thwarting the virus

Table 2
Summary of representative clinical trials of repurposed drugs.

Repurpose drug	Study Reference	Randomized Controlled Trial (Yes/No)	Number of Samples	Test group	Control group	Course of treatment (Number of Days)
RMD	Interventional [72]	Yes	1063	RMD	Placebo	10
Test group: 5						
RMD [73]	Interventional	Yes	397	RMD	RMD	Control
group: 10						
RMD	Interventional [74]	Yes	237	RMD	Placebo	10
RMD	Interventional [75]	No	53	RMD		10
HCQ + AZT [76]	Observational	No	1061	HCQ + AZT		HCQ: 10 AZT: 5
HCQ + AZT	Observational [77]	No	80	HCQ + AZT		3
HCQ + AZT [78]	Interventional	No	22	HCQ + AZT		Not mentioned
HCQ + AZT/ HCQ alone [79]	Observational	No	1438	HCQ alone + AZT, HCQ alone, AZT No drug		Not mentioned
HCQ + AZT/ HCQ alone [80]	Observational	No	490	HCQ + AZT	HCQ alone	Not Mentioned
Observational						
CQ, HCQ, AZTe/ LOP-RTO	No	120	CQ group		AZT group□LOP-RTO group	[81]
	HCQ	group				
HCQ	Observational [82]	No	1376	HCQ	Without HCQ	6
Basic treatments including						
HCQ [83]	Observational	No	550	HCQ	antiviral drugs and	7-10
antibiotics						
HCQ [84]	Observational	No	251	HCQ/AZT		Not mentioned

HCQ mentioned	Observational [85]	No	181	HCQ	Standard care without HCQ	Not
HCQ [86]	Interventional	Yes	150	HCQ + standard care	standard care	14~21
HCQ [87]	Observational	No	90	HCQ + AZT	HCQ alone	Not mentioned
HCQ	Interventional [77]	No	36	HCQ + AZT/ HCQ alone	Without HCQ	10
CQ, HCQ mentioned	Observational [88]	No	201	HCQ/CQ + AZT	HCQ alone, CQ alone	Not
HCQ [89]	Observational	No	11	HCQ + AZT		HCQ: 7 AZT: 4
Test group:						
CQ [90]	Interventional	Yes	81	High-dose CQ	low-dosage CQ	10Control
group: 5						
HCQ	Interventional [91]	Yes	30	HCQ + Standard care	standard care	5
CQ	Interventional [92]	Yes	22	CQ	LOP-RTO	10
Ribavirin + LOP-RTO, INF	Interventional [93]	Yes	127	βRibavirin -1b + LOP-RTO+ INF	LOP-RTO	14
β-1b						
LOP-RTO + atomized INF Not mentioned	Interventional [94]	No	13 2	LOP-RTO b and thymosin + atomized INFα1		α-
α-2b and thymosin α-1						
LOP-RTO, ARB LOP-RTO + treatment group	Observational Not mentioned [95]	No	178	ARB group, LOP-RTO group, combination therapy group		conventional
ARB						
LOP-RTO	Interventional [96]	Yes	199	LOP-RTO + standard care	standard care	14
LOP-RTO	Observational [97]	No	120	LOP-RTO	Without LOP-RTO	Not mentioned
LOP-RTO capsule interferon-α	Interventional + 7~10 [98]	Yes	60	Lianhua Qingwen capsule INF-α+LOP-RTO + Lianhua Qingwen		
LOP-RTO mentioned	Observational [99]	No	47	LOP-RTO medicine + adjuvant	adjuvant medicine	Not
LOP-RTO [100]	Observational	No	40	LOP-RTO		Not mentioned
LOP-RTO [101]	Observational	No	35	LOP-RTO		Not mentioned
LOP-RTO [102]	Observational	No	7	LOP-RTO		Not mentioned
LOP-RTO	Observational [103]	No	5	LOP-RTO	Without LOP-RTO	Not mentioned
FAV + INF-α	Interventional [104]	No	80	FAV + INF-α	LOP-RTO + interferon-α	14
ARB + INF-α [105]	Observational	No	141	ARB + INF-α	Interferon-α	10-14
ARB + Diammonium [106]	Observational	No	46	ARB glycyrrhizinate + diammonium		Not mentioned
Glycyrrhizinate						
ARB + LOP-RTO [107]	Observational	No	33	ARB + LOP-RTO	LOP-RTO	5-21

ARB [108]	Observational	No	190	ARB	Without ARB	Not mentioned	
ARB [100]	Observational	No	81	ARB	Without ARB	Not mentioned	
ARB	Interventional [109]	No	50	ARB	LOP-RTO	7	

(*continued on next page*)

Repurpose drug	Study	Randomized Controlled Trial (Yes/No)	Number of Samples	Test group	Control group	Course of treatment (Number of Days)	Reference
ARB + Lianhuaqingwen	Observational	No	32	ARB + lianhuaqingwen capsules	Lianhuaqingwen capsules	Not mentioned	[110]
ARB	Observational	No	20	ARB		6.7 days	[111]

Table 2 (*continued*)

Abbreviation: RMD: Remdesivir; CQ: Chloroquine; HCQ: Hydroxychloroquine; Arbidol: ARB; Azithromycin: AZT; Lopinavir: LOP; Ritonavir: RTO; Favipiravir: FAV;
QTc: corrected QT-interval; COVID-19: Coronavirus Disease 2019.

propagation and the overt host immune responses at the same time. The apparent side effects like photo sensitivity, gastrointestinal related symptoms, QT prolongation, and cardiomyopathy have raised questions regarding the safe use of CQ/HCQ in severely ill COVID-19 patients especially with co-morbidites. The clinical trials using CQ/HCQ alone or in combination have recommended a lower CQ dosage for critically ill COVID-19 patients because of its potential safety hazards, especially in combination with azithromycin and oseltamivir. Moreover, zinc additives have also been recommended to improve the efficacy of CQ and HCQ against SARS-CoV2. HCQ could be considered for the patients who cannot be administered RMD or when RMD is not available but with caution [71]. In conclusion, based on the results of ongoing /completed (Tables 2 and 3) clinical trials (151 trials) stringent parameters need to be considered to further support the usage of CQ and HCQ in COVID-19 patients. Our laboratory is actively engaged in elucidating the molecular pathways of therapeutic modalities used in malaria [62,112–115]. The effective nanoformulations of HCQ/CQ can help in reducing the side-effects of these wonder cost effective drugs for use in future dose-dosage regimens for COVID-19.

3.3. Noscapine

NOS, (Fig. 3) an opium alkaloid classically used as antitussive agent has been repositioned for its anti-tumor effect as it strongly binds to tubulin, arrests the cells in metaphase, induces apoptosis in the dividing human cells and has the advantage of large therapeutic window [116–122]. The antitussive effect of NOS is documented through suppression of bradykinin production or inhibition of its function through inhibition of B2 receptor thus preventing bronchoconstriction, mucous production and histamine release [123,124]. Bradykinin release has already been associated with concomitant increase in intracellular calcium and activation of cAMP signaling pathway to down regulate the protein abundance and TJ localization of Claudin 5, occludin and ZO-1. It is well documented that increased level of bradykinin is associated with inflammation and is a putative reason for dry cough response observed in patients [124]. Such imbalances in the bradykinin cellular level might be attributed to alteration in the functionality of its regulatory loop. ACE2 is a part of one such regulatory axis that reduces bradykinin's downstream metabolite viz. des-Arg bradykinin (DABK) by mediating its cleavage under normal cellular conditions [125]. A decrease in ACE2 expression has also been associated with increased DABK signaling. Thus, any alteration in ACE2 functionality can flare up bradykinin-DABK signaling axis associated responses [126]. Surprisingly, in COVID-19, a similar decrease in ACE2 functionality in infected cells has been observed that may be attributed to ACE2 shedding during virus spread [127]. Thus, subsequent loss of DABK regulatory loop may increase DABK cellular functionality and result in a pronounced bradykinin-DABK signaling associated inflammatory responses. Therefore, regulation of bradykinin signaling may be essential components of pharmaceuticals formulation to counter SARS-CoV2 virulence and pathogenesis.

Bradykinin, a nine aminoacid peptide is produced from HWMK when cleaved by plasma kallikrein. Any interruption in this process can result in alteration in bradykinin production in the cellular system. In lines with this, an interesting observation regarding NOS binding to HMWK has been speculated (Unpublished lab data). Formation of such NOS- HMWK complex may thus interfere with its processing through KL13 axis to produce bradykinin and DABK. Furthermore, HMWK also hinders contact activation of Factor XII [128] which is vital for hydrolysis of pre-kallikerin to product KL [129]. Therefore, NOS induced inhibition of HMWK processing into bradykinin might raise the physiological level of HMWK. It can be expected to decrease Factor-XII activation and which shall subsequently lead to defect in KL synthesis from prekallikerin, hence decreasing bradykinin synthesis. The restrained factor XII activation will decrease the coagulation responses and thus may be beneficial in improving the conditions of COVID-19 patients. Thus, NOS action in modulating the bradykinin-DABK may be an important factor for its success in dealing with SARS-CoV2 pathogenesis.

Additionally, the binding affinity of NOS and its analogues with protease of COVID-19 complex has recently been evaluated through MD simulations at different temperatures. It has been speculated NOS may exert its antiviral effects by inhibiting viral

protein synthesis [130]. Moreover, the well-versed role of NOS to disturb microtubule dynamics (anti-tubulin polymerisation activity) might also serve as important mode for meddling with the virus assembly [131]. Therefore, we propose that NOS may disperse S protein of SARS-CoV2 throughout the cytosol by interfering with the tubulin associated S protein transport. This event may attenuate incorporation of S protein in virion that can subsequently interfere with viral assembly and eventually viral load may decrease. Bradykinin is an important participant in thrombotic, inflammatory and renin angiotensin system. NOS has a substantial direct /indirect effect on all three systems in addition to its effect on microtubule dynamics and viral spread. Therefore, NOS can be an important drug candidate to combat COVID-19 for prophylactic and therapeutic interventions in near future.

3.4. Heparin sulphate

Heparin is a polydispersible, heterogenous natural product widely used as blood thinner. Unfractionated heparin, low molecular weight heparins and heparinoids are commonly employed clinically as anticoagulants due to excellent safety, bioavailability, stability and biopharmaceutical attributes. Several heparin analogues possessing antiviral activity are in clinical trials for coronaviridae and SARS associated coronavirus strain HSR1 [132], flaviviruses [133], herpes [123,134], influenza [135] and HIV [136,137]. Furthermore, heparan sulfate (HS), a glycosaminoglycan (GAG) member, binds to CoV surface proteins used by coronavirus for its attachment to target cells. Hence, binding of SARS-CoV-2 S1 RBD to heparin was studied using spectroscopic techniques and molecular modeling. A significant structural alteration is induced by heparin upon binding to SARS-CoV-2 S1 RBD. Further, heparin binding domains (moieties of basic amino acid residues) are solvent accessible on the SARS-CoV-2 S1 RBD surface and constitute a nonstop patch for convenient binding to heparin. Heparin is reported to inhibit SARS-associated coronavirus strain HSR1 cell invasion [133] and this supports the utilization of pharmaceutical heparins against SARS-associated coronavirus. Surface plasma resonance (SPR) studies elucidated that SARS-CoV-2 spike protein binds with higher affinity to heparin (K_D~55 nM) as compared to the receptor binding domain (RBD, K_D~1 μM) alone. An octasaccharide composed of IdoA2S-GlcNS6S could

Table 3
Summary of clinical observations and safety data of representative clinical trials Repurpose drug Inferences Safety Reference of repurposed drugs conducted in 2020.

Repurpose drug	Inferences	Safety	Reference
RMD	Shortening of recovery time of patients		[72]
RMD	Clinical improvement rate was not significantly different between control and test	QTc interval was prolonged especially in high transaminase level group	[73]
RMD	Clinical improvement rate was insignificant		[74]
RMD	Oxygen therapy requirements proportion was decreased in patients. Good clinical results and low mortality was seen in patients	QTc prolongation was severe in 12 % of the patients treated with combination therapywhich was higher than that caused by any single drug Adverse cardiac reactions in 103 cases were reported to be related only to HCQ (86 %) or to AZT (60 %). 17 reports (14 %) were related to LOP-RTO while CQ usage had 3 reports (2.5 %)	[75]
HCQ + AZT			[76]
HCQ + AZT			[77]
HCQ + AZT	Hospitalization time got lowered		[78]
HCQ + AZT/ HCQ alone			[79]
HCQ+ AZT/ HCQ alone	Insignificant difference in the mortality was seen in test v/s		[80]
CQ, HCQ, AZT, LOP-		QTc was prolonged and could not be shortened after treatment	

Table 3 (*continued*)

Repurpose drug	Inferences	Safety	Reference
	viral nucleic acid b/w experimental and control group		
HCQ		A prolonged QT interval after the use of HCQ which is greater after the addition of AZT	
HCQ	Decreased viral load		
CQ, HCQ		Although it is possible to prolong the QT interval, no death from TdP (Torsade de pointes) or arrhythmia has been reported	[87]
HCQ+ AZT	Post 9 day drug intervention, 8 patients tested –ve on RT-PCR test and 11 patients showed no severe or critical disease development	No obvious adverse reactions were observed	[77] [88] [89]
HCQ	Negative conversion rate of virus nucleic acid, body temperature's recovery time and adverse reactions were insignificantly different among control v/s test group	Adverse drug reactions such as diarrhea and abnormal liver functions were seen in 4 patients (26.7 %) under test group and 3 patients (20.0 %) in the control group. Difference among both group was insignificant ($p < 0.05$).	[90]
CQ			
CQ	Lung improvement rate and negative conversion rate of viral nucleic	High dose group had shown higher mortality as compared to low dose group. Five patients under CQ treated group shown mild adverse reactions, such as vomiting, abdominal	[91]

RTO	control group		[81]
HCQ			[82]
HCQ			[83]
HCQ	The intubation rate and mortality (test group v/s control) were significantly different		[84]
HCQ	Lower mortality, a shorter hospitalisation stay and decreased IL-6 levels were seen in experimental group		[85]
HCQ			[86]
	Severe conversion rate and overall survival proportion was not significantly different in test v/s control group Insignificant change in negative conversion rate of		

Table 3 (*continued*)

LOP-RTO		reactions increased to 45.45 % tested patients. 14 cases were cured and 1 case continued to be observed.	
LOP-RTO		Five patients among 7 tested had shown adverse drug reaction i.e abnormal liver function, diarrhea, decreased heart rate, hypertriglyceridemia etc.	[102]
	No difference in virus negative rate was seen between test and control group.		[103]
Favipiravir + INF-α	Virus clearance rate was higher in test group than control group The absorption rate of pneumonia in the test group was	A low level of adverse drug reactions were seen in test group	[104]
ARB + INF-α			[105]

Repurpose drug	Inferences	Safety	Reference
	Mortality, clinical improvement duration and virus not significantly different	Adverse drug rections were common among trial group	
LOP-RTO	Virus negative rate was shorter than	negative conversion rate and	[97]
Ribavirin + LOP-RTO, INF β-1b	acid was higher in experimental group than control group Shortening of virus	between the test group and the control group was	[92]
LOP-RTO+ Atomized INF α-2b and thymosin α1	negative rate in test group was observed No severe cases was seen and the clinical symptoms (such as fever, cough) were quickly improved, and the curative effect was remarkable		[93]
LOP-RTO, ARB, LOP-RTO+ ARB	Insignificant difference in viral nucleic acid negative conversion rate, symptom improvement rate and CT improvement rate was	Test groups have shown adverse reactions than the control group.	[94] [95]
LOP-RTO	observed between test group and control group, but deterioration rate was higher in treatment group		[96]
ARB+ Diammonium Glycyrrhizinate	Symptoms, lymphocytes, IL-6, albumin content and ALT were improved, and the overall cure rate and overall effective rate were higher.	ARB combined with diammonium glycyrrhizinate in the treatment of COVID-19 has less adverse reactions and high safety.	[106]
ARB+ LOP-RTO	CT improvement rate and virus negative conversion rate was faster in test group		[107]
ARB	A lower infection rate was seen in test group		[108]
ARB	Insignificant change in virus negative conversion rate and hospital stay was seen in test and control group	No death and any adverse reactions were recorded	[109]
ARB	Virus negative conversion rate was higher in experimental group	No adverse reactions seen	[110]
ARB + Lianhuaqingwen capsules	Body temperature recovery time improved, respiratory symptoms were cured, inflammation was lowered and hospitalisation time got shortened	Nausea, diarrhea and dizziness were not seen in all cases	[111]
ARB	An effective rate of 75 % was recorded. It was significantly effective in 45 %, effective in 30 %) and was ineffective in 25 %		[112]

(*continued on next page*)

Table 3 (*continued*)

Abbreviation: RMD: Remdesivir; CQ: Chloroquine; HCQ: Hydroxychloroquine; Arbidol: ARB; AZ Azithromycin: AZT; Lopinavir: LOP; Ritonavir: RTO; QTc: corrected QT-interval; COVID-19: Coronavirus Disease 2019.

inhibit spike-heparin interaction with an IC50 of 38 nM [138].

Therefore, heparin may prove beneficial in patients with COVID-19, however, accurate adjustment of heparin dose is a matter of concern for this drug. In an uncontrolled retrospective study, Tang and colleagues recently described that LMWH helped in reducing mortality in patients with COVID-19 with coagulopathy when compared to those who did not receive heparin. Although the molecular mechanism for this benefit is not completely understood, it seems likely that the anti-inflammatory effects of heparin are contributory. However, the most efficacious ratio of anticoagulant to anti-inflammatory activity of heparin is still to be determined [139,140].

4. Miscellaneous options

4.1. Vitamin C and Vitamin D

	control group		
LOP-RTO	Effectiveness rate was higher in test group as compare to control group.		[98]
LOP-RTO	Stability of body temperature and blood biochemistry profile was better in experimental groups as compate to control groupThe changes of body temperature, blood routine and blood biochemistry in the experimental group were better than those in the control group	Adverse drug reactions were not observed	[99]
LOP-RTO	Moderate drug reactions occurred in 29 cases (72.5 %), 2 or more adverse reactions occurred in 13 cases (32.5%), and severe reactions were seen in 4 cases (10%). Elevated triglyceride (50%), nausea (17.5%), diarrhoea (17.5%) were recorded as adverse drug reactions. Female and male groups were indistinguishable for adverse drug reactions.		[100]
LOP-RTO	Diarrhea as adverse drug		[101]

concentrations was found to protect lung epithelium [141,142]. Owing to virus internalization and spread, the cytokine cascade is activated, and infilterating neutrophils in the lungs destroy alveolar capillaries. Vitamin C indirectly regulates the alveolar fluid accumulation by attenuating the activation and prevention of neutrophils infilteration. In addition, it is postulated that vitamin C prevents the formation of neutrophil extracellular traps, a biological event of neutrophil associated vascular injury. Therefore, vitamin C should be investigated under controlled randomized clinical trials for the prevention of viral pneumonia in COVID-19 [143].

Apart from vitamin C, vitamin D supplementation is a useful approach to reduce risk by inducing cathelicidins and defensins. Vitamin D lowers viral replication rates and also controls the pro-inflammatory cytokines production responsible for lung injuries. Observational studies and clinical trials have reported that vitamin D supplementation can manage the risk of influenza [144]. The 1,25-dihydroxyvitamin D, an active metabolite of vitamin D exhibited the inhibitory effect in human nasal epithelial cells infected with SARS-CoV-2 [145].

4.2. Immunotherapy

Middle East respiratory syndrome coronavirus (MERS-CoV) employed its S proteins as an adhesion molecule to augment host entry through a receptor called dipeptidyl peptidase-4 (DPP4). Dipeptidyl peptidase-4 (DPP4) is considered a key player in the transmission of signals and triggering of innate and

Vitamin C modulates the susceptibility to a variety of bacterial and viral infections, influences the immune system and at high adaptive immune responses. Hence, designing monoclonal antibodies may be a great and prolific approach as compared to vaccine development to exhibit the immediate protective effect. It has been demonstrated that m336, a human monoclonal antibody extracted from the phage display library binds to the receptor- binding region of S protein of MES coronavirus and exhibits strong neutralization intensity to MERS-CoV [146]. Furthermore, m336 also decreases the viral RNA titer in lungs by 40,000–90,000 folds [147]. Injections of monoclonal antibody m336 or hyperimmune plasma against MERS-CoV when administered to infected monkeys resulted in considerable reduction in symptoms of clinical disease however; viral load in lungs was reduced considerably only in hyperimmune plasma group. Both hyperimmune plasma and m336 therapy exhibited promising results in terms of mitigating the symptoms however; none of them completely prevented them [148]. H Mab m336 significantly decreased the viral RNA titers in addition to viral-associated pathological alterations in lung tissue of rabbit [149]. S nanoparticles in mice yielded high-level neutralizing antibodies against homologous viruses [150]. Similar to the concept of using neutralising Ab, a plasma therapy (using plasma of recovered COVID-19 patients) based approach is also gaining momentum and is already approved for treating COVID-19 patients in some cases [151].

5. Challenges and opportunities

The exponential surge in COVID-19 cases has put forth numerous challenges for every affected nation

but has also demonstrated some promises. The knowledge gained by the scientific community during first SARS-COV pandemic in 2003 has been quite useful to deal with the current pandemic. RMD, an antiviral drug previously developed for Ebola disease has shown a great promise in treating COVID-19 patients. Although approximately 2/3 of patients treated with this drug were cured, well designed studies are required to confirm its suitability in management of COVID-19. Repeated use of RMD may lead to development of resistance in SARS-COV2 strain which can be of prime concern in near future. HCQ is already being recommended for clinical use in selective patients. However the side-effects associated with its use are alarming and require further investigation. This creates further opportunities to identify additional therapeutic approaches that can be helpful in such scenarios. As proposed, NOS is one such drug which can be effective in managing the viral spread. Additionally, combinatorial approaches can be of use to decrease the toxicity related issues. Most importantly, to prevent the viral spread, immunotherapy options are also depicting great potential. However, one of biggest problem associated with this is the rapidly mutating nature of the virus. It can be seen that the virus is rapidly evolving and mutating at an unprecedented rate. This may render the vaccination approaches non-functional in near future. Therefore, alternative therapeutic strategies in addition to the classical drug modalities will play important role to deal resurgence of epidemic in times to come.

6. Conclusion

The development of new drugs as well as vaccination and immunotherapy stretegies require indepth knowledge about structural biology, genomic and proteomic signatures of SARS-CoV2. The research in these areas might be affected by the recombination potential of CoV well as the complex multiorgan sequalae including the effect on the immune system. The identification of already available drugs for their possible use in halting the spread and resurgence of COVID 19 can be the most plausible approach to follow till the dedicated therapeutic and immunization agents against SARS-CoV2 are available.

Clinical significance

COVID-19 is spreading at a rapid pace. Therefore, momentum to open new frontiers for fight against its spread is the need of hour. The repurposing of already available drugs or their combinations as well as adjunct therapies are widely acclaimed pharmaceutical approaches that can aid in the functional recovery and clinical management of COVID-19 patients. Their probable mechanisms of actions and their status in clinical trials are also the case of discussion in this text.

Declaration of Competing Interest

None of the authors has conflict of interest with this submission.

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References

[1] E. Prompetchara, C. Ketloy, T. Palaga, Immune responses in COVID-19 and potential vaccines: lessons learned from SARS and MERS epidemic, Asian Pac. J. Allergy Immunol. 38 (2020) 1–9.
[2] R. Lu, X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, W. Wang, et al., Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding, Lancet 395 (2020) 565–574.
[3] T.T.Y. Lam, M.H.H. Shum, H.C. Zhu, Y.G. Tong, X.B. Ni, Y.S. Liao, W. Wei, et al., Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins, Nature 583 (2020) 282–285.
[4] T. Hampton, Bats may be SARS reservoir, JAMA 294 (2005) 2291.
[5] W. Li, Z. Shi, M. Yu, W. Ren, C. Smith, J.H. Epstein, H. Wang, et al., Bats are natural reservoirs of SARS-like coronaviruses, Science 310 (2005) 676–679.
[6] A. Banerjee, K. Kulcsar, V. Misra, M. Frieman, K. Mossman, Bats and Coronaviruses, Viruses11, 2019, p. 41.
[7] P. Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.R. Si, et al., A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature 579 (2020) 270–273.
[8] J.F.W. Chan, S. Yuan, K.H. Kok, K.K.W. To, H. Chu, J. Yang, F. Xing, et al., A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster, Lancet 395 (2020) 514–523.
[9] M. Giovanetti, D. Benvenuto, S. Angeletti, M. Ciccozzi, The first two cases of 2019-CoV in Italy: Where they come from? J. Med. Virol. 92 (2020) 518–521.
[10] D. Paraskevis, E.G. Kostaki, G. Magiorkinis, G. Panayiotakopoulos, G. Sourvinos, S. Tsiodras, Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event, Infect. Genet. Evol. 79 (2020) 104212.
[11] P. Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.R. Si, et al., A new coronavirus of probable bat origin, Nature 579 (2020) 270–273.
[12] N. Doremalen, T. Bushmake, D.H. Morris, M.G. Holbrook, A. Gamble, B. N. Williamson, A. Tamin, Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1, New Engl. J. Med. 382 (2020) 16.
[13] A. Sharma, S. Tiwari, M.K. Deb, J.L. Marty, Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2): a global pandemic and treatment strategies, Int. J. Antimicrob. Agents 56 (2020) 106054.
[14] S.Y. Ren, W.B. Wang, Y.G. Hao, H.R. Zhang, Z.C. Wang, Y.L. Chen, R.D. Gao, Stability and infectivity of coronaviruses in inanimate environments, World J. Clin. Cases 8 (2020) 1391–1399.
[15] Y. Chen, Y. Guo, P. Pan, Z.J. Zhao, Structure analysis of the receptor binding of 2019-nCoV, Biochem. Biophys. Res. Commun. 525 (2020) 135–140.
[16] M. Letko, A. Marzi, V. Munster, Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses, Nature Microbiol. 5 (2020) 562–569.
[17] R. Yan, Y. Zhang, Y. Li, L. Xia, G. Guo, Q. Zhou, Structural basis for recognition of SARS-COV-2 by full-length human ACE2, Science 367 (2020) 1444–1448.
[18] A.C. Walls, Y.J. Park, M.A. Tortorici, A. Wall, A.T. McGuire, D. Veesler, Structure, function, and antigenicity of the SARSCoV-2 spike glycoprotein, Cell 180 (2020) 1–12.
[19] J.A. Schalken, A.J. Roebroek, P.P. Oomen, S.S. Wagenaar, F.M. Debruyne, H.

P. Bloemers, W.J. Van de Ven, Fur gene expression as a discriminating marker for small cell and nonsmall cell lung carcinomas, J. Clin. Invest. 80 (1987) 1545–1549.
[20] K. Hatsuzawa, M. Hosaka, M. Nagase, A. Shoda, Structure and expression of mouse furin, a yeast Kex2-related protease. Lack of processing of coexpressed prorenin in GH4C1 cells, J. Biol. Chem. 265 (1990) 22075–22078.
[21] H. Wang, P. Yang, K. Liu, F. Guo, Y. Zhang, G. Zhang, C. Jiang, SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway, Cell Res. 18 (2008) 290–301.
[22] A. Heurich, H. Hofmann-Winkle, S. Giere, T. Liepold, O. Jahn, S. Pohlmann, TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein, J. Virol. 88 (2014) 1293–1307.
[23] B.J. Bosch, Cathepsin L functionally cleaves the Severe Acute Respiratory Syndrome Coronavirus Class I fusion protein upstream of rather than adjacent to the fusion peptide, J. Virol. 82 (2008) 8887–8890.
[24] W.F. Lima, T.P. Prakash, H.M. Murray, G.A. Kinberger, W. Li, A.E. Chappell, C.
S. Li, S.F. Murray, H. Gaus, P.P. Seth, E.E. Swayze, S.T. Crooke, Single-stranded siRNAs activate RNAi in animals, Cell 150 (2012) 883–894.
[25] K. Ui-Tei, K. Nishi, T. Takahashi, T. Nagasawa, Thermodynamic control of small RNA-mediated gene silencing, Frontiers Genet. 3 (2012) 101.
[26] L. Cui, H. Wang, Y. Ji, J. Yang, S. Xu, X. Huang, Z. Wang, L. Qin, P. Tien, X. Zhou, D. Guo, Y. Chen, The nucleocapsid protein of coronaviruses acts as a viral suppressor of RNA silencing in mammalian cells, J. Virol. 89 (2015) 9029–9043.
[27] A. Hegyi, J. Ziebuhr, Conservation of substrate specificities among coronavirus main proteases, J. Gen. Virol. 83 (2002) 595–599.
[28] E. Prentice, J. McAuliffe, X. Lu, K. Subbarao, M.R. Denison, Identification and characterization of severe acute respiratory syndrome coronavirus replicase proteins, J. Virol. 78 (2004) 9977–9986.
[29] W.J. Guan, Z.Y. Ni, Y. Hu, W.H. Liang, C.Q. Ou, J.X. He, L. Liu, et al., Clinical characteristics of coronavirus disease 2019 in China, N. Engl. J. Med. 382 (2020) 1708–1720.
[30] L. Subissi, C.C. Posthuma, A. Collet, J.C. Zevenhoven-Dobbe, A.E. Gorbalenya, E. Decroly, E.J. Snijder, et al., One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities, PNAS (2014) E3900–E3909.
[31] A.O. Adedeji, B. Marchand, A.J.W. Velthuis, E.J. Snijde, S. Weiss, R.L. Eoff, K. Singh, S.G. Sarafianos, Mechanism of nucleic acid unwinding by SARS-CoV helicase, PLoS One 7 (2012) e36521.
[32] M. Bouvet, A. Lugari, C.C. Posthuma, J.C. Zevenhoven, S. Bernard, S. Betzi, L. Imbert, et al., CoronavirusNsp10, a critical Co-factor for activation of multiple replicative enzymes, J. Biol. Chem. 289 (2014) 25783–25796.
[33] R.L. Graham, A.C. Sims, S.M. Brockway, R.S. Baric, M.R. Denison, The nsp2 replicase proteins of murine hepatitis virus and Severe Acute Respiratory Syndrome Coronavirus are dispensable for viral replication, J. Virol. 79 (2005) 13399–13411.
[34] M.G. Wathelet, M. Orr, M.B. Frieman, R.S. Baric, Severe Acute Respiratory Syndrome coronavirus evades antiviral signaling: role of nsp1 and rational design of an attenuated strain, J. Virol. 81 (2007) 11620–11633.
[35] X. Cao, M.A. Surma, K. Simons, Polarized sorting and trafficking in epithelial cells, Cell Res. 22 (2012) 793–805.
[36] A.T. Rudiger, P. Mayrhofer, Y. Ma-Lauer, G. Pohlentz, J. Muthing, A. Brunn, A. von, C. Chwegmann-Weßels, Tubulins interact with porcine and human S proteins of the genus Alphacoronavirusand support successful assembly and release of infectious viral particles, Virol. 497 (2016) 185–197.
[37] X. Zhao, F. Guo, M.A. Comunale, A. Mehta, M. Sehgal, P. Jain, A. Cuconati, L. Hanxin, M.B. Timothy, C. Jinhong, J.T. Guo, Inhibition of endoplasmic reticulum-resident glucosidases impairs Severe Acute Respiratory Syndrome Coronavirus and Human Coronavirus NL63 spike protein-mediated entry by altering the glycan processing of angiotensin I-converting enzyme 2, Antimicrob. Agents Chemother. 59 (2014) 206–216.
[38] X. Lu, J. Pan, J. Tao, D. Guo, SARS-CoV nucleocapsid protein antagonizes IFNbresponse by targeting initial step of IFN-binduction pathway, and its C-terminal region is critical for the antagonism, Virus Genes 42 (2011) 37–45.
[39] Y. Hu, L. Wei, G. Ting, C. Yan, J. Yanwen, L. Ping, M. Quingjun, L. Xuan, C. Cheng, The severe acute respiratory syndrome coronavirus nucleocapsid inhibits type I interferon production by interfering with TRIM25-Mediated RIG-I ubiquitination, J. Virol. 91 (2017) e02143–16.
[40] F. Zheng, C. Liao, Q. Fan, et al., Clinical characteristics of children with coronavirus disease 2019 in Hubei, China, Current Med. Sci. 40 (2020) 1–6.
[41] J. Klumperman, J.K. Locker, A. Meijer, M.C. Horzinek, H.J. Geuze, P.J. Rottier, Coronavirus M proteins accumulate in the Golgi complex beyond the site of virion budding, J. Virol. 68 (1994) 6523–6534.
[42] B.G. Hogue, C.E. Machamer, Coronavirus structural proteins and virus assembly, in: S. Perlman, T. Gallagher, E.J. Snijder (Eds.), Nidoviruses., ASM Press, Washington, DC, USA, 2008, pp. 179–200.
[43] Y. Huang, Z. Yang, W. Kong, G. Nabel, Generation of synthetic severe acute respiratory syndrome coronavirus pseudoparticles: implications for assembly and vaccine production, J. Virol. (2004) 12557–12565.
[44] H. Luo, D. Wu, C. Shen, K. Chen, X. Shen, Severe acute respiratory syndrome coronavirus membrane protein interacts with nucleocapsid protein mostly through their carboxyl termini by electrostatic attraction, Int. J. Biochem. Cell Biol. 38 (2006) 589–599.
[45] J. Torres, K. Parthasarathy, X. Lin, R. Saravanan, A. Kukol, D. Liu, Model of a putative pore: the pentameric alpha-helical bundle of SARS coronavirus E protein in lipid bilayers, Biophys. J. 91 (2006) 938–947.
[46] K. Parthasarathy, L. Ng, X. Lin, D. Liu, K. Pervushin, X. Gong, J. Torres, Structural flexibility of the pentameric SARS coronavirus envelopeprotein ion channel, Biophys. J. 95 (2008) L39–L41.
[47] J.L. Nieto-Torres, C. Verdia-´ Baguena, J.M. Jimenez-Guard´ eno, J.A. Regla-Nava,˜ C. Castano-Rodriguez, C. Fernandez-Delgado, J. Torres, et al., Severe acute˜ respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome, Virol. 485 (2015) 330–339.
[48] M. DeDiego, J. Nieto-Torres, J. Regla-Nava, J. Jimenez-Guardeno, R. Fernandez-˜ Delgado, C. Fett, C. Castano-Rodriguez, S. Perlman, L. Enjuanes, Inhibition of NF-˜ kappaB mediated inflammation in severe acute respiratory syndome coronavirus- infected mice increases survival, J. Virol. 88 (2014) 913–924.
[49] A.L. Odegard, M.H. Kwan, E. Walukiewicz, M. Banerjee, A. Schneemann, J. E. Johnson, Low endocytic pH and capsid protein autocleavage are critical components of flock house virus cell entry, J. Virol. 83 (2009) 8628–8637.
[50] A. Milewska, K. Falkowski, K. Magdalena, et al., Kallikrein 13: a new player in coronaviral infections, bioRxiv (2020).
[51] L. Sodhi, C. Wohlford-Lenane, Y. Yamaguchi, Attenuation of pulmonary ACE 2 activity impairs inactivation of des-arg9 bradykinin/BKB1R axis and facilitates LPS induced neutrophil infiltration, Am. J. Physiol. Lung Cell Mol. Physiol. 314 (2018) L17–29.
[52] F. van de Veerdonk, M.G. Netea, M. van Deuren, J.W.M. van der Meer, Q. de Mast, R.J. Bruggemann, H. van der Hoeven, Kinins and cytokines in COVID-19: a comprehensive pathophysiological approach, Preprints (2020) 2020040023.
[53] K. Hwa, W. Lin, Y. Hou, T. Yeh, Peptide mimicrying between SARS coronavirus spike protein and human proteins reacts with SARS patient serum, J. Biomed. Biotechnol. (2008). Article ID 326464.
[54] F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, J. Xiang, Y. Wang, et al., Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study, Lancet 395 (2020) 1054–1062.
[55] C. Mycroft-West, D. Su, S. Elli, S. Guimond, The 2019 coronavirus (SARS-CoV-2) surface protein (Spike) S1 Receptor Binding Domain undergoes conformational change upon heparin binding, bioRxiv (2020).
[56] C. Li, L. Wang, L. Ren, Antiviral mechanisms of candidate chemical medicines and traditional Chinese medicines for SARS-CoV-2 infection, Virus Res. 286 (2020) 198073.
[57] J.S. Khalili, H. Zhu, N.S.A. Mak, Y. Yan, Y. Zhu, Novel coronavirus treatment with ribavirin: groundwork for an evaluation concerning COVID-19, J. Med. Virol. 92 (2020) 740–746.
[58] T.K. Warren, R. Jordan, M.K. Lo, A.S. Ray, R.L. Mackman, V. Soloveva, D. Siegel, et al., Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys, Nature 531 (2016) 381–385.

[59] M.L. Holshue, C. DeBolt, S. Lindquist, K.H. Lofy, J. Wiesman, H. Bruce, C. Spitters, et al., First case of 2019 novel coronavirus in the United States, N. Engl. J. Med. 382 (2020) 929–936.
[60] M. Wang, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, et al., Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro, Cell Res. 30 (2020) 269–271.
[61] A.M. Hashem, B.S. Alghamdi, A.A. Algaissi, et al., Therapeutic use of chloroquine and hydroxychloroquine in COVID-19 and other viral infections: a narrative review, Travel Med. Infect. Dis. 35 (2020) 101735.
[62] A. Kashyap, R. Kaur, A. Baldi, U.K. Jain, R. Chandra, J. Madan, Chloroquine diphosphate bearing dextran nanoparticles augmented drug delivery and overwhelmed drug resistance in Plasmodium falciparum parasites, Int. J. Biol. Macromol. 114 (2018) 161–168.
[63] N. Gupta, S. Agrawal, P. Ish, Chloroquine in COVID-19: the evidence, Monaldi Arch. Chest Dis. 90 (2020) 177–178.
[64] A.M. Hashem, B.S. Alghamdi, A.A. Algaissi, Therapeutic use of chloroquine and hydroxychloroquine in COVID-19 and other viral infections: a narrative review, Travel Med. Infect. Dis. 35 (2020) 101735.
[65] R.E. Ferner, J.K. Aronson, Chloroquine and hydroxychloroquine in covid-19, BMJ 369 (2020) m1432.
[66] J. Gao, Z. Tian, X. Yang, Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies, Biosci. Trends 14 (2020) 72–73.
[67] M.J. Vincent, E. Bergeron, S. Benjannet, B.R. Erickson, P.E. Rollin, T.G. Ksiazek, N.G. Seidah, S.T. Nichol, Chloroquine is a potent inhibitor of SARS coronavirus infection and spread, Virol. J. 2 (2005) 69.
[68] K. Kupferschmidt, J. Cohen, Race to find COVID-19 treatments accelerates, Science 367 (2020) 1412–1413.
[69] V. Gies, N. Bekaddour, Y. Dieudonn´e, A. Guffroy, Q. Frenger, F. Gros, M. P. Rodero, J.P. Herbeuval, A.S. Korganow, Beyond anti-viral effects of chloroquine/hydroxychloroquine, Front. Immunol. (2020).
[70] A.K. Chakrabarty, K. Saha, S. Chopra, P. Sen, Effects of chloroquine on the serum complement system, Immunopharmacology 10 (1985) 111–118.
[71] M. Oostra, C. de Haan, R.J. de Groot, P.J.M. Rottier, Glycosylation of the severe acute respiratory syndrome coronavirus triple-spanning membrane proteins 3a and M, J. Virol. 80 (2006) 2326–2336.
[72] R. Humeniuk, A. Mathias, H. Cao, et al., Safety, tolerability, and pharmacokinetics of remdesivir, an antiviral for treatment of COVID-19, in healthy subjects, Clin. Transl. Sci. (2020) (in press).
[73] J.H. Beigel, K.M. Tomashek, L.E. Dodd, et al., Remdesivir for the treatment of Covid-19 -Preliminary report, N. Engl. J. Med. (2020) (in press).
[74] J.D. Goldman, D.C.B. Lye, D.S. Hui, et al., Remdesivir for 5 or 10 days in patients with Sever Covid-19, N. Engl. J. Med. (2020) (in press).
[75] Y. Wang, D. Zhang, G. Du, et al., Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial, Lancet 395 (2020) 1569–1578.
[76] J. Grein, N. Ohmagari, D. Shin, et al., Compassionate use of remdesivir for patients with Severe Covid-19, N. Engl. J. Med. 382 (2020) 2327–2336.
[77] M. Million, J.C. Lagier, P. Gautret, et al., Early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: a retrospective analysis of 1061 cases in Marseille, France, Travel Med. Infect. Dis. 35 (2020) 101738.
[78] P. Gautret, J.C. Lagier, P. Parola, et al., Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial, Int. J. Antimicrob. Agents 56 (2020) 105949.
[79] A. Cipriani, A. Zorzi, D. Ceccato, et al., Arrhythmic profile and 24-hour QT interval variability in COVID-19 patients treated with hydroxychloroquine and azithromycin, Int. J. Cardiol. 316 (2020) 280–284.
[80] E.S. Rosenberg, E.M. Dufort, T. Udo, et al., Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York State, JAMA 323 (2020) 2493–2502.
[81] A. Ramireddy, H. Chugh, K. Reinier, et al., Experience with hydroxychloroquine and azithromycin in the COVID-19 pandemic: implications for qt interval monitoring, J. Am. Heart Assoc. 9 (2020) e017144.
[82] A. Gerard, S. Romani, A. Fresse, et al., Off-label" use of hydroxychloroquine,´ azithromycin, lopinavir-ritonavir and chloroquine in COVID-19: a survey of cardiac adverse drug reactions by the French Network of Pharmacovigilance Centers, Therapie 75 (2020) 371–379.
[83] J. Geleris, Y. Sun, J. Platt, et al., Observational study of hydroxychloroquine in hospitalized patients with Covid-19, N. Engl. J. Med. 382 (2020) 2411–2418.
[84] B. Yu, C. Li, P. Chen, et al., Low dose of hydroxychloroquine reduces fatality of critically ill patients with COVID-19, Sci. China Life Sci. (2020) (in press).
[85] E. Chorin, L. Wadhwani, S. Magnani, et al., QT interval prolongation and Torsade De Pointes in patients with COVID-19 treated with hydroxychloroquine/ azithromycin, Heart Rhythm 17 (2020) 1425–1433.
[86] M. Mahevas, V.T. Tran, M. Roumier, et al., Clinical efficacy of´ hydroxychloroquine in patients with covid-19 pneumonia who require oxygen: observational comparative study using routine care data, BMJ 369 (2020) m1844.
[87] N.J. Mercuro, C.F. Yen, D.J. Shim, et al., Risk of QT interval prolongation associated with use of hydroxychloroquine with or without concomitant azithromycin among hospitalized patients testing positive for coronavirus disease 2019 (COVID-19), JAMA Cardiol. 5 (2020) 1036–1041.
[88] M. Saleh, J. Gabriels, D. Chang, et al., The effect of chloroquine, hydroxychloroquine and azithromycin on the corrected QT interval in patients with SARS-COV-2 infection, Circ. Arrhythm. Electrophysiol. 13 (2020) e008662.
[89] M. Wu, B. Wang, P. Liu, et al., Efficacy and safety of hydroxychloroquine combined with azithromycin in treatment of patients with refractory common coronavirus disease-19(COVID-19), Acad. J. Second Mil. Med. Univ. 13 (2020) e008662.
[90] J. Chen, D. Liu, L. Liu, et al., A pilot study of hydroxychloroquine in treatment of patients with moderate COVID-19, Zhejiang Da Xue Xue Bao Yi Xue Ban 49 (2020) 215–219.
[91] M.G.S. Borba, F.F.A. Val, V.S. Sampaio, et al., Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) infection: a randomized clinical trial, JAMA Netw Open. 3 (2020) e208857.
[92] M. Huang, T. Tang, P. Pang, et al., Treating COVID-19 with chloroquine, J. Mol. Cell Biol. 12 (2020) 322–325.
[93] I.F. Hung, K.C. Lung, E.Y. Tso, et al., Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial, Lancet 395 (2020) 1695–1704.
[94] H. Zheng, H. Su, S. Wang, et al., Thirteen cases of COVID patients treated by the combination of lopinavir/ritonavir tablets, atomized interferon α-2b and thymosin α1, Clinical Res. Practice 5 (2020) 36–38.
[95] C.Y. Wen, Z.W. Xie, Y.P. Li, et al., Real-world efficacy and safety of lopinavir/ ritonavir and arbidol in treating with COVID-19 : an observational cohort study, Zhonghua Nei Ke Za Zhi 59 (2020) E012.
[96] B. Cao, Y. Wang, D. Wen, et al., A trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19, N. Engl. J. Med. 382 (2020) 1787–1799.
[97] D. Yan, X.Y. Liu, Y.N. Zhu, et al., Factors associated with prolonged viral shedding and impact of Lopinavir/Ritonavir treatment in hospitalised non-critically ill patients with SARS-CoV-2 infection, Eur. Respir. J. 56 (2020) 2000799.
[98] S. Wang, H. Wang, H. Chen, et al., Lianhua Qingwen capsule and interferon-α combined with lopinavir/ritonavir for the treatment of 30 COVID-19 patients, J. Bengbu Med. Coll. 45 (2020) 154–155.
[99] X.T. Ye, Y.L. Luo, S.C. Xia, et al., Clinical efficacy of lopinavir/ritonavir in the treatment of Coronavirus disease 2019, Eur. Rev. Med. Pharmacol. Sci. 24 (2020) 3390–3396.
[100] L. Liang, T. Zheng, Q. Wang, et al., Safety Analysis of lopinavir/ritonavir tablets in 40 hospitalized patients with Coronavirus Disease 2019, Chin. J. Hosp. Pharm (2020) (in press).
[101] S. Yang, J. Liang, T. Liu, et al., Analysis of diarrhea associated with lopinavir- ritonavir for COVID-19 and its prevention, Med. J. West China 32 (2020) 485–488.
[102] Y. Xu, L. Shen, W. Han, et al., Pharmaceutical care of lopinave/litonawe in the treatment of critically coronavirus disease 2019, Chin. J. Clin. Pharmacol. Ther. (2020) (in press).

[103] C.Y. Cheng, Y.L. Lee, C.P. Chen, et al., Lopinavir/ritonavir did not shorten the duration of SARS CoV-2 shedding in patients with mild pneumonia in Taiwan, J. Microbiol. Immunol. Infect. 53 (2020) 488–492.
[104] Q. Cai, M. Yang, D. Liu, et al., Experimental treatment with favipiravir for COVID- 19: an open-label control study, Engineering (Beijing) (2020) (in press).
[105] P. Xu, J. Huang, Z. Fan, et al., Arbidol/IFN-α2b therapy for patients with Corona Virus Disease 2019: a retrospective multicenter cohort study, Microbes Infect. 22 (2020) 200–205.
[106] J. Xi, S. Xiang, H. Zhang, et al., Clinical observation of arbidol combined with diammonium glycyrrhizinate in the treatment of COVID-19, Chin. J. Hosp. Pharm (2020) (in press).
[107] L. Deng, C. Li, Q. Zeng, et al., Arbidol combined with LPV/r versus LPV/r alone against Corona Virus Disease 2019: a retrospective cohort study, J. Infect. 81 (2020) e1–e5.
[108] J.N. Zhang, W.J. Wang, B. Peng, et al., Potential of arbidol for post-exposure prophylaxis of COVID-19 transmission-A preliminary report of a retrospective cohort Study, Curr. Med. Sci. 40 (2020) 480–485.
[109] Z. Zhu, Z. Lu, T. Xu, et al., Arbidol monotherapy is superior to lopinavir/ritonavir in treating COVID-19, J. Infect. 81 (2020) e21–e23.
[110] L. Liu, L. Yuan, Y. Feng, et al., Clinical study on combined scheme of Lianhuaqingwen Capsules and abidole in the treatment for coranavirus disease 2019, Guangdong Med. J. (2020) (in press).
[111] J. Zang, F. Peng, Y. Jiang, et al., Retrospective clinical study of efficacy and safety of arbidol hydrochloride in the treatment of corona virus disease 2019 with common type, Clin. Med. J. 18 (2020) 26–29.
[112] P. Gautret, J.C. Lagier, P. Parola, V.T. Hoang, L. Meddeb, M. Mailhe, B. Doudier, et al., Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial, Int. J. Antimicrob. Agents (2020) 105949.
[113] A. Kalra, M.L. Dubey, N.K. Ganguly, K. Mohan, R.C. Mahajan, Effect of nifedipine treatment on oxidative metabolism of peritoneal macrophages and neutrophils of Plasmodium berghei-infected mice, Exp. Parasitol. 74 (1992) 20–26.
[114] A. Kalra, M.L. Dubey, N.K. Ganguly, R.C. Mahajan, Effect of nifedipine on calcium status and chemiluminescence response of phagocytes during Plasmodium berghei infection in mice, J. Pharm. Pharmacol. 45 (1993) 540–544.
[115] A. Kalra, M.L. Dubey, N.K. Ganguly, K. Mohan, R.C. Mahajan, Altered course of Plasmodium berghei infection by nifedipine treatment, APMIS 101 (1993) 667–671.
[116] J. Madan, N. Dhiman, V.K. Parmar, S. Sardana, P.V. Bharatam, R. Aneja, R. Chandra, A. Katyal, Inclusion complexes of noscapine in beta-cyclodextrin offer better solubility and improved pharmacokinetics, Cancer Chemother. Pharmacol. 65 (2010) 537–548.
[117] J. Madan, N. Dhiman, S. Sardana, R. Aneja, R. Chandra, A. Katyal, Long- circulating poly(ethylene glycol)-grafted gelatin nanoparticles customized for intracellular delivery of noscapine: preparation, in-vitro characterization, structure elucidation, pharmacokinetics, and cytotoxicity analyses, Anticancer Drugs 22 (2011) 543–555.
[118] J. Madan, R.S. Pandey, V. Jain, O.P. Katare, R. Chandra, A. Katyal, Poly (ethylene)-glycol conjugated solid lipid nanoparticles of noscapine improve biological half-life, brain delivery and efficacy in glioblastoma cells, Nanomedicine 9 (2013) 492–503.
[119] R. Chandra, J. Madan, P. Singh, A. Chandra, P. Kumar, V. Tomar, S.K. Dass, Implications of nanoscale based drug delivery systems in delivery and targeting tubulin binding agent, noscapine in cancer cells, Curr. Drug Metab. 13 (2012) 1476–1483.
[120] V. Jain, B. Jain, P. Tiwari, J. Saini, U.K. Jain, R.S. Pandey, M. Kumar, O.P. Katare, R. Chandra, J. Madan, Nanosolvated microtubule-modulating chemotherapeutics: a case-to-case study, Anticancer Drugs 24 (2013) 327–336.
[121] K. Jyoti, K. Kaur, R.S. Pandey, U.K. Jain, R. Chandra, J. Madan, Inhalable nanostructured lipid particles of 9-bromo-noscapine, a tubulin-binding cytotoxic agent: in vitro and in vivo studies, J. Colloid Interface Sci. 445 (2015) 219–230.
[122] K. Kaur, R.K. Sodhi, A. Katyal, R. Aneja, U.K. Jain, O.P. Katare, J. Madan, Wheat germ agglutinin anchored chitosan microspheres of reduced brominated derivative of noscapine ameliorated acute inflammation in experimental colitis, Colloids Surf. B Biointerfaces 132 (2015) 225–235.
[123] V. Tomar, S. Kukreti, S. Prakash, J. Madan, R. Chandra, Noscapine and its analogs as chemotherapeutic agent: current updates, Curr. Top. Med. Chem. 17 (2017) 174–188.
[124] E. Trybala, J.A. Liljeqvist, B. Svennerholm, T. Bergstrom, Herpes simplex virus types 1 and 2 differ in their interaction with heparan sulfate, J. Virol. 74 (2000) 9106–9114.
[125] P.C.J. Rida, D. LiVecche, A. Ogden, J. Zhou, R. Aneja, The noscapine chronicle: a pharmaco-historic biography of the opiate alkaloid family and its clinical applications, Med. Res. Rev. 35 (2015) 1072–1096.
[126] C. Vickers, P. Hales, V. Kaushik, L. Dick, J. Gavin, J. Tang, K. Godbout, T. Parsons, et al., Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase, J. Biol. Chem. 277 (2002) 14838–14843.
[127] S.A. Ebrahimi, Noscapine, a possible drug candidate for attenuation of cytokine release associated with SARS-CoV-2, Drug Dev. Res. (2020) (in press).
[128] I. Glowacka, S. Bertram, P. Herzog, S. Pfefferle, I. Steffen, M.O. Muench, et al., Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63, J. Virol. 84 (2010) 1198–1205.
[129] F. Espana, O.D. Ratnoff, The role of prekallikrein and high-molecular-weight kininogen in the contact activation of Hageman factor (factor XII) by sulfatides and other agents, J. Lab. Clin. Med. 102 (1983) 487–499.
[130] K. Chatterjee, J.L. Thornton, J.W. Bauer, E.A. Vogler, C.A. Siedlecki, Moderation of prekallkrein-factor XII interactions in surface activation of coagulation by protein-adsorption competition, Biomaterials 30 (2009) 4915–4920.
[131] D. Kumar, K. Kumari, A. Jayaraj, V. Kumar, R.V. Kumar, S.K. Dass, R. Chandra, P. Singh, Understanding the binding affinity of noscapines with protease of SARS- CoV-2 for COVID-19 using MD simulations at different temperatures, J. Biomol. Struct. Dyn. (2020) (in press).
[132] J. Lang, N. Yang, J. Deng, K. Liu, P. Yang, G. Zhang, C. Jiang, Inhibition of SARS pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans, PLoS One 6 (2011) e23710.
[133] A. Milewska, M. Zarebski, P. Nowak, K. Stozek, J. Potempa, K. Pyrc, Human coronavirus NL63 utilizes heparan sulfate proteoglycans for attachment to target cells, J. Virol. 88 (2014) 13221–13230.
[134] E. Vicenzi, I. Pagani, S. Ghezzi, S.L. Taylor, T.R. Rudd, M.A. Lima, M.A. Skidmore, E.A. Yates, Subverting the mechanisms of cell death: flavivirus manipulation of host cell responses to infection, Biochem. Soc. Trans. 46 (2018) 609–617.
[135] D. WuDunn, P.G. Spear, Initial interaction of herpes simplex virus with cells is binding to heparan sulfate, J. Virol. 63 (1989) 52–58.
[136] M.A. Skidmore, A. Kajaste-Rudnitski, N.M. Wells, S.E. Guimond, T.R. Rudd, E. A. Yates, E. Vicenzi, Inhibition of influenza H5N1 invasion by modified heparin derivatives, Med. Chem. Res. 6 (2015) 640–646.
[137] M. Rusnati, D. Coltrini, P. Oreste, G. Zoppetti, A. Albini, D. Noonan, F. d'Adda di Fagagna, M. Giacca, M. Presta, Interaction of HIV-1 Tat protein with heparin, Role of the backbone structure, sulfation, and size, J. Biol. Chem. 272 (1997) 11313–11320.
[138] H.A. Harrop, C.C. Rider, Heparin and its derivatives bind to HIV-1 recombinant envelope glycoproteins, rather than to recombinant HIV-1 receptor, CD4. Glycobiol. 8 (1998) 131–137.
[139] L. Liu, P. Chopra, X. Li, M.A. Wolfert, S.M. Tompkins, G.J. Boons, SARS-CoV-2 spike protein binds heparan sulfate in a length- and sequence-dependent manner, bioRxiv. (2020) (in press).
[140] N. Tang, H. Bai, X. Chen, J. Gond, D. Li, Z. Sun, Anticoagulant treatment is associated with decrease mortality in severe coronavirus disease 2019 patients with coagulopathy, J. Thromb. Haemost. 18 (2020) 1094–1099.
[141] H. Hemila, Vitamin C and SARS coronavirus, J. Antimicrob. Chemother. 52 (2003) 1049–1050.
[142] A.A. Fowler, J.D. Truwit, R.D. Hite, P.E. Morris, C. DeWilde, A. Priday, B. Fisher, et al., Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory

failure: the Citris-Ali randomized clinical trial, JAMA 322 (2019)
1261–1270.
[143] Z.Y. Peng, Vitamin C Infusion for the Treatment of Severe 2019-nCoV Infected Pneumonia, 2020. https://clinicaltrials.gov/ct2/show/NCT04264533.
[144] W.B. Grant, H. Lahore, S.L. McDonnell, C.A. Baggerly, C.B. French, J.L. Aliano, H. P. Bhattoa, Evidence that vitamin D supplementation could reduce risk of influenza and COVID-19 infections and deaths, Nutrients 12 (2020) 988.
[145] A.R. Martineau, N.G. Forouhi, Vitamin D for COVID-19: a case to answer? Lancet Diabetes Endocrinol. 8 (2020) 735–736.
[146] T. Ying, L. Du, T.W. Ju, P. Prabakaran, C.C.Y. Lau, L. Lu, Q. Liu, L. Wang, Y. Feng, et al., Exceptionally potent neutralization of Middle East respiratory syndrome coronavirus by human monoclonal antibodies, J. Virol. 88 (2014) 7796–7805. [147] K.V. Houser, L. Gretebeck, T. Ying, Y. Wang, L. Vogel, E.W. Lamirande, K.
W. Bock, et al., Prophylaxis with a middle East Respiratory Syndrome Coronavirus (MERS-CoV)-specific human monoclonal antibody protects rabbits from MERS-CoV infectionm, J. Infect. Dis. 213 (2016) 1557–1561.
[148] N. van Doremalen, D. Falzarano, T. Ying, E. de Wit, T. Bushmaker, et al., Efficacy of antibody-based therapies against Middle East respiratory syndrome coronavirus (MERS-CoV) in common marmosets, Antiviral Res. 143 (2017)
30–37.
[149] C.M. Coleman, Y.V. Liu, H. Mu, J.K. Taylor, M. Massare, D.C. Flyer, G.E. Smith, M. B. Frieman, Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice, Vaccine 32 (2014) 3169–3174.
[150] E. De Clercq, Antivirals and antiviral strategies, Nat. Rev. Microbiol. 2 (2004)
704–720.
[151] V. Wiwanitkit, Convalescent plasma therapy in the treatment of COVID-19: some considerations: correspondence, Int. J. Surg. 80 (2020) 26.